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hereby certify that the annexed is a true copy of the Provisional specification in
connection with Application No. PP 0323 for a patent by INTERNATIONAL
DIABETES INTSITUTE and DEAKIN UNIVERSITY filed on 11 November 1997.

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day of November 1998

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A U S T R A L I A
Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"A Novel Gene and Uses Therefor-II"

The invention is described in the following statement:

- 1A -

A NOVEL GENE AND USES THEREFOR-II

FIELD OF THE INVENTION

5 The present invention relates generally to a nucleic acid molecule which encodes a protein associated with the modulation of obesity, diabetes and metabolic energy levels. More particularly, the present invention is directed to a nucleic acid molecule and a recombinant and purified naturally occurring protein encoded thereby and their use in therapeutic and diagnostic protocols for conditions such as obesity, diabetes and energy imbalance. The subject nucleic acid
10 molecule and protein and their derivatives, homologues, analogues and mimetics are proposed as therapeutic and diagnostic agents for obesity, diabetes and energy imbalance.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a
15 stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Bibliographic details of the publications referred by author in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and
20 amino acid sequences referred to in the specification are defined following the bibliography.

BACKGROUND OF THE INVENTION

The increasing sophistication of recombinant DNA technology is greatly facilitating research and
25 development in the medical, veterinary and allied human and animal health fields. This is particularly the case in the investigation of the genetic bases involved in the etiology of certain disease conditions. One particularly significant condition from the stand point of morbidity and mortality is obesity and its association with non-insulin-dependent diabetes mellitus (NIDDM) and cardiovascular disease.

30

Obesity is defined as a pathological excess of body fat and is the result of an imbalance between

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energy intake and energy expenditure for a sustained period of time. Obesity is the most common metabolic disease found in affluent societies. The prevalence of obesity in these nations is alarmingly high, ranging from 10% to upwards of 50% in some subpopulations (Bouchard, 1994). Of particular concern is the fact that the prevalence of obesity appears to be rising consistently in affluent societies and is now increasing rapidly in less prosperous nations as they become more affluent and/or adopt cultural practices from the more affluent countries (Zimmet, 1992).

In Australia, for example, studies using the definition of obesity of BMI>30 have found prevalence rates for obesity of 8.2-9.3% in men and 9.1-11.1% in women (Risk Factor Prevalence Study Management Committee, 1990; Waters and Bennett 1995). The prevalence rates for obesity are increasing in Australia, as they are in many affluent societies. Bennett and Magnus (1994) found that the mean weight of Australian females aged 20-69 increased by 3.1 kg (from 61.7 to 64.8 kg) from 1980 to 1989, while the corresponding increase in males was 1.8 kg (from 77.0 to 78.8 kg). No change in height was observed during this period. Accordingly, the crude prevalence rates of obesity increased from 8.0 to 13.2% in females and from 9.3 to 11.5% in males (Bennett and Magnus 1994). All of the above changes were statistically significant ($p<0.05$).

The high and increasing prevalence of obesity has significant health implications. Obesity has been identified as a key risk indicator of preventable morbidity and mortality due to disease such as NIDDM and cardiovascular disease (National Health and Medical Research Council, 1996). The annual costs of obesity in Australia, for example, associated with these and other disease conditions have been conservatively estimated at AU\$810 million (National Health and Medical Research Council, 1996).

A genetic basis for the etiology of obesity is indicated *inter alia* from studies in twins, adoption studies and population-based analyses which suggest that genetic effects account for 25-80% of the variation in body weight in the general population (Bouchard 1994; Kopelman *et al*, 1994; Ravussin, 1995). It is considered that genes determine the possible range of body weight in an individual and then the environment influences the point within this range where the individual

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is located at any given time (Bouchard, 1994).

Obesity is a complex and heterogeneous disorder and of considerable relevance to society. However, despite numerous studies into genes thought to be involved in the pathogenesis of obesity, there have been surprisingly few significant findings in this area. In addition, genome-wide scans in various population groups have not produced definitive evidence of the chromosomal regions having a major effect on obesity.

The hypothalamus has long been recognised as a key brain area in the regulation of energy intake. Early studies led to the dual-centre hypothesis which proposed that two opposing centres in the hypothalamus were responsible for the initiation and termination of eating, the lateral hypothalamus (LHA; "hunger centre") and ventromedial hypothalamus (VMH; "satiety centre"; Stellar 1954). The dual-centre hypothesis has been repeatedly modified to accommodate the increasing information about the roles played by various other brain regions, neurotransmitter systems, and hormonal and neural signals originating in the gut on the regulation of food intake. In addition to the LHA and VMH, the paraventricular nucleus (PVN) is now considered to have an important integrative function in the control of energy intake.

A large number of neurotransmitters have been investigated as possible hypothalamic regulators of feeding behaviour including neuropeptide Y (NPY), glucagon-like peptide 1 (GLP-1), melanin-concentrating hormone (MCH), serotonin, cholecystokinin and galanin. Some of these neurotransmitters stimulate food intake, some act in an anorexigenic manner and some have diverse effects on energy intake depending on the site of administration. For example, gamma-aminobutyric acid (GABA) inhibits food intake when injected into the LHA, but stimulates eating when injected into the VMH or PVN (Leibowitz, 1985). Feeding behaviour is thought to be greatly influenced by the interaction of stimulatory and inhibitory signals in the hypothalamus.

In work leading up to the present invention, the inventors have made a significant break through in determining a genetic basis of obesity by identifying a genetic sequence differentially expressed in lean and obese animals. In accordance with the present invention, the inventors have isolated a novel gene which is proposed to be associated with energy balance and also in modulating

obesity and diabetes.

SUMMARY OF THE INVENTION

5 One aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a protein or a derivative, homologue, analogue or mimetic thereof wherein said nucleic acid molecule is expressed in larger amounts in hypothalamus tissue of obese animals compared to lean animals.

10 Another aspect of the present invention is directed to a nucleic acid molecule comprising a nucleotide sequence, or a complementary form thereof, encoding an amino acid sequence substantially as set forth in SEQ ID NO:2 or an amino acid sequence having at least 30% similarity to all or a part thereof or a mimetic or said amino acid sequence or a nucleotide sequence-capable of hybridizing to said nucleic acid molecule under low stringency conditions
15 at 42°C and wherein said nucleic acid molecule is expressed in a larger amount in hyperthalamus tissue of obese animals compared to lean animals.

Yet another aspect of the present invention provides a nucleic acid molecule comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein or a
20 derivative, homologue, analogue or mimetic thereof wherein said nucleotide sequence is as substantially set forth in SEQ ID NO:1 or a nucleotide sequence having at least about 30% similarity to all or part of SEQ ID NO:1 and/or is capable of hybridizing to SEQ ID NO:1 under low stringency conditions at 42°C and wherein said nucleic acid molecule is expressed in a larger amount in hyperthalamus tissue of obese animals compared to lean animals.

25

Still yet another aspect of the present invention provides an isolated protein or a derivative, homologue, analogue or mimetic thereof which is produced in a larger amount in hyperthalamus tissue of obese animals compared to lean animals.

30 In yet another aspect of the present invention, there is provided an isolated protein or a derivative, homologue, analogue or mimetic thereof wherein said protein comprises an amino

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acid sequence substantially as set forth in SEQ ID NO:2 or an amino acid sequence having at least 30% similarity to all or part of SEQ ID NO:2 and wherein said protein is produced in a larger amount in hyperthalamus tissue of obese animals compared to lean animals.

- 5 A further aspect of the present invention is directed to an isolated protein or a derivative, homologue, analogue or mimetic thereof wherein said protein is encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:1 or a nucleotide sequence having at least 60% similarity to all or part of SEQ ID NO:1 and/or is capable of hybridizing to SEQ ID NO:1 under low stringency conditions at 42°C.

10

The protein of the present invention is referred to as "beacon" and the nucleotide sequence encoding beacon is referred to as the *beacon* gene.

- A further aspect of the present invention relates to a composition comprising beacon or its derivatives, homologues, analogues or mimetics or agonists or antagonists of beacon together with one or more pharmaceutically acceptable carriers and/or diluents.

- Yet a further aspect of the present invention contemplates a method for treating a subject comprising administering to said subject a treatment effective amount of beacon or a derivative, homologue, analogue or mimetic thereof or a genetic sequence encoding same or an agonist or antagonist of beacon or *beacon* gene expression for a time and under conditions sufficient to effect treatment.

- In accordance with this and other aspects of the present invention, treatments contemplated herein include but are not limited to obesity, anorexia, weight maintenance, energy imbalance and diabetes. Treatment may be by the administration of a pharmaceutical composition or genetic sequences *via* gene therapy. Treatment is contemplated for human subjects as well as animals such as animals important to livestock industry.

- 30 Still yet another aspect of the present invention is directed to a diagnostic agent for use in monitoring or diagnosing conditions such as but not limited to obesity, anorexia, weight

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maintenance, energy imbalance and/or diabetes, said diagnostic agent selected from an antibody to beacon or its derivatives, homologues, analogues or mimetics and a genetic sequence useful in PCR, hybridization, RFLP amongst other techniques.

- 5 A summary of SEQ ID NOs used throughout the subject specification is provided in Table 1.

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TABLE 1

	SEQ ID NO.	DESCRIPTION
	1	Nucleotide sequence for <i>beacon</i>
5	2	Amino acid sequence for beacon
	3	Complementary sequence for SEQ ID NO:1
	4	Primer sequence
	5	Primer sequence
	6	Primer sequence
10	7	Primer sequence
	8	Primer sequence
	9	Primer sequence

A summary of the single and three letter abbreviations for amino acid residues used in the present specification is provided in Table 2.

15

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TABLE 2

5	Amino Acid	Three-letter Abbreviation	One-letter Symbol
	Alanine	Ala	A
	Arginine	Arg	R
	Asparagine	Asn	N
10	Aspartic acid	Asp	D
	Cysteine	Cys	C
	Glutamine	Gln	Q
	Glutamic acid	Glu	E
	Glycine	Gly	G
15	Histidine	His	H
	Isoleucine	Ile	I
	Leucine	Leu	L
	Lysine	Lys	K
	Methionine	Met	M
20	Phenylalanine	Phe	F
	Proline	Pro	P
	Serine	Ser	S
	Threonine	Thr	T
	Tryptophan	Trp	W
25	Tyrosine	Tyr	Y
	Valine	Val	V
	Any residue	Xaa	X

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a representation showing the nucleotide sequence of both strands of a differentially expressed band in hypothalamus tissue of lean and obese *Psammomys obesus* corresponding to
5 *beacon*. The amino acids encoded by each codon are shown above in single letter code and the numbering refers to the amino acid position from the start codon.

Figure 2 is a representation showing (A). Amino acid alignments of beacon with putative human, mouse, *Caenorhabditis elegans*, *Fasciola hepatica*, rice and *Saccharomyces cerevisiae* gene
10 products. (B). Amino acid alignments of beacon with human ubiquitin and ubiquitin-like protein 8 from *Arabidopsis thaliana*. Identical amino acids are marked with a line and plus signs indicate deletions are indicated by forward slashes. A spliced leader sequence in the *F. hepatica* gene did not allow the aminoterminal amino acids to be compared.

15 **Figure 3** is a graphical representation showing correlations of hypothalamic beacon gene expression with (A) body weight and (B) log plasma insulin concentrations in *Psammomys obesus*.

Figure 4 is a graphical representation showing improved correlations of hypothalamic *beacon*
20 gene expression with (A) body weight and (B) log plasma insulin concentrations in leptin-treated *Psammomys obesus*.

Figure 5 is a graphical representation showing correlations of beacon gene expression in adipose tissue with (A) body weight and (B) log plasma insulin, and in liver with (C) body weight and
25 (D) log plasma insulin.

Figure 6 is a graphical representation showing effects of leptin treatment on hypothalamic *beacon* gene expression in lean and obese *Psammomys obesus*.

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Figure 7 is a graphical representation showing effects of leptin treatment on adipose tissue *beacon* gene expression in lean and obese *Psammomys obesus*. (*p=0.014 compared with lean control animals).

- 5 **Figure 8** is a graphical representation showing effects of nicotine treatment on (A) hypothalamic and (B) adipose tissue *beacon* gene expression in lean and obese *Psammomys obesus*.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is predicated in part on the identification of a novel gene associated *inter alia* with regulation of energy balance obesity and diabetes. The gene was identified following
5 differential screening of hypothalamic mRNA between lean and obese animals.

Accordingly, one aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotide encoding or complementary to a sequence encoding a protein or a derivative homologue, analogue or mimetic thereof wherein said nucleic acid molecule is
10 expressed in a larger amount in hypothalamus tissue of obese animals compared to lean animals.

The terms "lean" and "obese" are used in their most general sense but should be considered relative to the standard criteria for determining obesity. Generally, for human subjects the definition of obesity is BMI>30 (Risk Factor Prevalence 1990; Waters and Bennett, 1995).
15

Conveniently, an animal model may be employed to study the effects of obese and lean animals. In particular, the present invention is exemplified using the *Psammomys obesus* (the Israeli sand rat) animal model of dietary-induced obesity and NIDDM. In its natural desert habitat, an active lifestyle and saltbush diet ensure that they remain lean and normoglycemic (Shafrir and Gutman,
20 1993). However, in a laboratory setting on a diet of *ad libitum* chow (on which many other animal species remain healthy), a range of pathophysiological responses are seen (Barnett *et al*, 1994a, b; Barnett *et al*, 1995). By the age of 16 weeks, more than half of the animals become obese and approximately one third develop NIDDM. Only hyperphagic animals go on to develop hyperglycemia, highlighting the importance of excessive energy intake in the pathophysiology
25 of obesity and NIDDM in *Psammomys obesus* (Collier *et al*, 1997a; Walder *et al*, 1997a). Other phenotypes found include hyperinsulinemia, dyslipidemia and impaired glucose tolerance (Collier *et al*, 1997a, b). *Psammomys obesus* exhibit a range of bodyweight and blood glucose and insulin levels which forms a continuous curve that closely resembles the patterns found in human populations, including the inverted U-shaped relationship between blood glucose and insulin
30 levels known as "Starling's curve of the pancreas" (Barnett *et al*, 1994a; DeFronzo, 1988). It is the heterogeneity of the phenotypic response of *Psammomys obesus* which make it an ideal

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model to study the etiology and pathophysiology of obesity and NIDDM.

A preferred embodiment of the present invention is directed to a nucleic acid molecule comprising a nucleotide sequence or a complementary form thereof encoding an amino acid sequence substantially as set forth in SEQ ID NO:2 or an amino acid sequence having at least 60% similarity to all or a part thereof or is a mimetic thereof or a nucleotide sequence capable of hybridizing to said nucleic acid molecule under low stringency conditions at 42°C and wherein said nucleic acid molecule is expressed in larger amounts in hyperthalamus tissue of obese animals compared to lean animals.

10

Another embodiment of the present invention provides a nucleic acid molecule comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein or a derivative, homologue, analogue or mimetic thereof wherein said nucleotide sequence is as substantially set forth in SEQ ID NO:1 or a nucleotide sequence having at least about 60% similarity to all or part of SEQ ID NO:1 and/or is capable of hybridizing to SEQ ID NO:1 under low stringency conditions at 42°C and wherein said nucleic acid molecule is expressed in a larger amount in hyperthalamus tissue of obese animals compared to lean animals.

Reference herein to similarity is generally at a level of comparison of at least 15 consecutive or substantially consecutive nucleotides or at least 5 consecutive or substantially consecutive amino acid residues.

The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels.

30

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1%

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v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and
5 from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

10

The nucleotide sequence or amino acid sequence of the present invention may correspond to exactly the same sequence of the naturally occurring gene (or corresponding cDNA) or protein or may carry one or more nucleotide or amino acid substitutions, additions and/or deletions. The nucleotide sequence set forth in SEQ ID NO:1 corresponds to a new gene referred to herein as
15 "*beacon*". The corresponding protein is "*beacon*". Reference herein to *beacon* includes, where appropriate, reference to the genomic gene or cDNA as well as any naturally occurring or induced derivatives. Apart from the substitutions, deletions and/or additions to the nucleotide sequence, the present invention further encompasses mutants, fragments, parts and portions of the nucleotide sequence corresponding to *beacon*.

20

A homologue is considered to be a *beacon* gene from another animal species. The *beacon* gene is exemplified herein from rat hypothalamus. The invention extends, however, to the homologous gene, as determined by nucleotide sequence and/or function, from humans, primates, livestock animals (eg. cows, sheep, pigs, horses, donkeys), laboratory test animals (eg.
25 mice, guinea pigs, hamsters, rabbits), companion animals (eg. cats, dogs) and captured wild animals (eg. rodents, foxes, deer, kangaroos).

The nucleic acid of the present invention and in particular *beacon* and its derivatives and homologues may be in isolated or purified form and/or may be ligated to a vector such as an
30 expression vector. Expression may be in a eukaryotic cell line (eg. mammalian, insect or yeast cells) or in microbial cells (eg. *E. coli*) or both.

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The derivatives of the nucleic acid molecule of the present invention include oligonucleotides, PCR primers, antisense molecules, molecules suitable for use in co-suppression and fusion nucleic acid molecules. Ribozymes and DNA enzymes are also contemplated by the present invention directed to *beacon* or its mRNA. Derivatives and homologues of *beacon* are
5 conveniently encompassed by those nucleotide sequences capable of hybridizing to SEQ ID NO:1 or 3 under low stringency conditions at 42°C.

Another aspect of the present invention provides an isolated protein or a derivative, homologue, analogue or mimetic thereof which is produced in larger amounts in hyperthalamus tissue in
10 obese animals compared to lean animals.

In a more preferred aspect of the present invention, there is provided an isolated protein or a derivative, homologue, analogue or mimetic thereof wherein said protein comprises an amino acid sequence substantially as set forth in SEQ ID NO:2 or an amino acid sequence having at
15 least 60% similarity to all or part of SEQ ID NO:2 and wherein said protein is produced in larger amounts by hyperthalamus tissue of obese animals compared to lean animals.

A further aspect of the present invention is directed to an isolated protein or a derivative, homologue, analogue or mimetic thereof wherein said protein is encoded by a nucleotide
20 sequence substantially as set forth in SEQ ID NO:1 or a nucleotide sequence having at least 60% similarity to all or part of SEQ ID NO:1 and/or is capable of hybridizing to SEQ ID NO:1 under low stringency conditions at 42°C.

The protein of this aspect of the present invention is *beacon*.
25

Reference herein to *beacon* includes reference to isolated or purified naturally occurring *beacon* protein molecules as well as any derivatives, homologues, analogues and mimetics thereof. Derivatives includes parts, fragments and portions of *beacon* as well as single and multiple amino acid substitutions, deletions and/or additions to *beacon*. A derivative of *beacon* is conveniently
30 encompassed by molecules encoded by a nucleotide sequence capable of hybridizing to SEQ ID NO:1 under low stringency conditions at 42°C.

Other derivatives of beacon include chemical analogues. Analogues of beacon contemplated herein include, but are not limited to, modifications to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use
5 of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde
10 followed by reduction with NaBH_4 ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with NaBH_4 .

15

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation
20 followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted
25 maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or
30 alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form

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a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

5

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of
10 amino acids. A list of unnatural amino acid, contemplated herein is shown in Table 3.

TABLE 3

	Non-conventional amino acid	Code	Non-conventional amino acid	Code
5				
	α -aminobutyric acid	Abu	L-N-methylalanine	Nmala
	α -amino- α -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
	aminocyclopropane- carboxylate	Cpro	L-N-methylasparagine	Nmasn
			L-N-methylaspartic acid	Nmasp
10	aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
	aminonorbomyl- carboxylate	Norb	L-N-methylglutamine	Nmgln
			L-N-methylglutamic acid	Nmglu
	cyclohexylalanine		Chexa L-N-methylhistidine	Nmhis
	cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
15	D-alanine	Dal	L-N-methylleucine	Nmleu
	D-arginine	Darg	L-N-methyllysine	Nmlys
	D-aspartic acid	Das	L-N-methylmethionine	Nmmet
	D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
	D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
20	D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
	D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
	D-isoleucine	Dile	L-N-methylproline	Nmpro
	D-leucine	Dleu	L-N-methylserine	Nmser
	D-lysine	Dlys	L-N-methylthreonine	Nmthr
25	D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
	D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
	D-phenylalanine	Dphe	L-N-methylvaline	Nmval
	D-proline	Dpro	L-N-methylethylglycine	Nmetg
	D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
30	D-threonine	Dthr	L-norleucine	Nle
	D-tryptophan	Dtrp	L-norvaline	Nva

	D-tyrosine	Dtyr	α -methyl-aminoisobutyrate	Maib
	D-valine	Dval	α -methyl- γ -aminobutyrate	Mgab
	D- α -methylalanine	Dmala	α -methylcyclohexylalanine	Mchexa
	D- α -methylarginine	Dmarg	α -methylcyclopentylalanine	Mcpen
5	D- α -methylasparagine	Dmasn	α -methyl- α -naphthylalanine	Manap
	D- α -methylaspartate	Dmasp	α -methylpenicillamine	Mpen
	D- α -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- α -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D- α -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
10	D- α -methylisoleucine	Dmile	N-amino- α -methylbutyrate	Nmaabu
	D- α -methylleucine	Dmleu	α -naphthylalanine	Anap
	D- α -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- α -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D- α -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
15	D- α -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- α -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- α -methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D- α -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D- α -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
20	D- α -methyltyrosine	Dmtty	N-cyclodecylglycine	Ncdec
	D- α -methylvaline	Dmval	N-cylcododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
25	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
30	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
	D-N-methylleucine	Dnmleu	N-(3-indolylethyl)glycine	Nhtrp

	D-N-methyllysine	Dnmlys	N-methyl- γ -aminobutyrate	Nmgabu
	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmt
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpn
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
5	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyl- α -naphthylalanine	Nmanap
10	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	γ -aminobutyric acid	Gabu	N-(<i>p</i> -hydroxyphenyl)glycine	Nhtyr
	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L- α -methylalanine	Mala
15	L- α -methylarginine	Marg	L- α -methyloasparagine	Masn
	L- α -methyloaspartate	Masp	L- α -methyl- <i>t</i> -butylglycine	Mtbug
	L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
	L- α -methylglutamine	Mgln	L- α -methylglutamate	Mglu
	L- α -methylhistidine	Mhis	L- α -methylhomophenylalanine	Mhphe
20	L- α -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
	L- α -methyllucine	Mleu	L- α -methyllysine	Mlys
	L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle
	L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
	L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
25	L- α -methylserine	Mser	L- α -methylthreonine	Mthr
	L- α -methyltryptophan	Mtrp	L- α -methyltyrosine	Mtyr

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L- α -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhpe
N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe
carbamylmethyl)glycine		carbamylmethyl)glycine	
1-carboxy-1-(2,2-diphenyl-	Nmbc		
5 ethylamino)cyclopropane			

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having (CH₂)_n spacer groups with n=1 to n=6, 10 glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C α and N α -methylamino acids, introduction of double bonds between C α and C β atoms of amino acids and 15 the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

All such modifications may also be useful in stabilizing the beacon molecule for use in *in vivo* 20 administration protocols or for diagnostic purposes.

The identification of beacon permits the generation of a range of therapeutic molecules capable of modulating expression of beacon or modulating the activity of beacon. Modulators contemplated by the present invention includes agonists and antagonists of beacon expression. 25 Antagonists of beacon expression include antisense molecules, ribozymes and co-suppression molecules. Agonists include molecules which increase promoter activity or which interfere with negative regulatory mechanisms. Antagonists of beacon include antibodies and inhibitor peptide fragments. All such molecules may first need to be modified to enable such molecules to penetrate cell membranes. Alternatively, viral agents may be employed to introduce genetic 30 elements to modulate expression of *beacon*. In so far as beacon acts in association with other genes such as the *ob* gene which encodes leptin, the therapeutic molecules may target both the

beacon and *ob* genes or their translation products.

The present invention contemplates, therefore, a method for modulating expression of *beacon* in a mammal, said method comprising contacting the *beacon* gene with an effective amount of
5 a modulator of *beacon* expression for a time and under conditions sufficient to up-regulate or down-regulate or otherwise modulate expression of *beacon*. For example, a nucleic acid molecule encoding *beacon* or a derivative or homologue thereof may be introduced into a cell to enhance the ability of that cell to produce beacon, conversely, *beacon* antisense sequences such as oligonucleotides may be introduced to decrease the availability of beacon molecules.

10

Another aspect of the present invention contemplates a method of modulating activity of beacon in a mammal, said method comprising administering to said mammal a modulating effective amount of a molecule for a time and under conditions sufficient to increase or decrease beacon activity. The molecule may be a proteinaceous molecule or a chemical entity and may also be
15 a derivative of beacon or its ligand.

Modulating levels of *beacon* expression is important in the treatment of a range of conditions such as obesity, anorexia, energy imbalance and diabetes. It may also be useful in the agricultural industry to assist in the generation of leaner animals, or where required, more obese
20 animals. Accordingly, the mammal contemplated by the present invention includes but is not limited to humans, primates, livestock animals (eg. pigs, sheep, cows, horses, donkeys), laboratory test animals (eg. mice, rats, guinea pigs, hamsters, rabbits), companion animals (eg. dogs, cats) and captured wild animals (eg. foxes, kangaroos, deer). A particularly preferred host is a human, primate or livestock animal.

25

Accordingly, the present invention contemplates in one embodiment a composition comprising a modulator of *beacon* expression or beacon activity and one or more pharmaceutically acceptable carriers and/or diluents. In another embodiment, the composition comprises beacon or a derivative, homologue, analogue or mimetic thereof and one or more pharmaceutically
30 acceptable carriers and/or diluents. The compositions may also comprise leptin or modulations of leptin activity or *ob* expression.

For brevity, all such components of such a composition are referred to as "active components".

The compositions of active components in a form suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous
5 preparation of sterile injectable solutions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi.

10 The carrier can be a solvent or other medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils.

The preventions of the action of microorganisms can be brought about by various antibacterial
15 and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

20

Sterile injectable solutions are prepared by incorporating the active components in the required amount in the appropriate solvent with optionally other ingredients, as required, followed by sterilization by, for example, filter sterilization, irradiation or other convenient means. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods
25 of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When *beacon* and beacon including beacon itself are suitably protected they may be orally
30 administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may

be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The
5 percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μ g and 2000 mg of active
10 compound.

The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such
15 as magnesium stearate; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with
20 shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and
25 formulations.

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Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active component may be compounded for convenient and effective administration in sufficient amounts with a suitable pharmaceutically acceptable carrier in dosage unit form. A unit dosage form can, for example, contain the principal active component in amounts ranging from 0.5 μ g to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 μ g to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

In general terms, effective amounts of beacon will range from 0.01 ng/kg/body weight to above 10,000 mg/kg/body weight. Alternative amounts range from 0.1 ng/kg/body weight to above 1000 mg/kg/body weight.

The pharmaceutical composition may also comprise genetic molecules such as a vector capable

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of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating *beacon* expression or beacon activity. The vector may, for example, be a viral vector.

- 5 Still another aspect of the present invention is directed to antibodies to beacon and its derivatives and homologues. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to beacon or may be specifically raised to beacon or derivatives or homologues thereof. In the case of the latter, beacon or its derivatives or homologues may first need to be associated with a carrier molecule. The antibodies and/or
- 10 recombinant beacon or its derivatives of the present invention are particularly useful as therapeutic or diagnostic agents.

For example, beacon and its derivatives can be used to screen for naturally occurring antibodies to beacon which may occur in certain autoimmune diseases or where cell death is occurring.

- 15 These may occur, for example in some autoimmune diseases. Alternatively, specific antibodies can be used to screen for beacon. Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA.

- Antibodies to beacon of the present invention may be monoclonal or polyclonal and may be
- 20 selected from naturally occurring antibodies to the beacon or may be specifically raised to the beacon or its derivatives. In the case of the latter, the beacon protein may need first to be associated with a carrier molecule. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include
- 25 fragments and hybrids of antibodies. The antibodies of this aspect of the present invention are particularly useful for immunotherapy and may also be used as a diagnostic tool or as a means for purifying beacon.

For example, specific antibodies can be used to screen for beacon proteins. The latter would be important, for example, as a means for screening for levels of beacon in a cell extract or other biological fluid or purifying beacon made by recombinant means from culture supernatant fluid. Techniques for the assays contemplated herein are known in the art and include, for
5 example, sandwich assays and ELISA.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used
10 with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of beacon.

Both polyclonal and monoclonal antibodies are obtainable by immunization with the enzyme or protein and either type is utilizable for immunoassays. The methods of obtaining both types
15 of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of beacon, or antigenic parts thereof, collecting serum from the animal, and isolating specific sera by any of the known immunoabsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the
20 potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell
25 line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art. (See, for example Douillard and Hoffman, Basic Facts about Hybridomas, in *Compendium of Immunology* Vol II, ed. by Schwartz, 1981; Kohler and Milstein, *Nature* 256: 495-499, 1975; *European Journal of Immunology* 6: 511-519, 1976).

30

Another aspect of the present invention contemplates a method for detecting beacon in a

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biological sample from a subject said method comprising contacting said biological sample with an antibody specific for beacon or its derivatives or homologues for a time and under conditions sufficient for an antibody-beacon complex to form, and then detecting said complex.

- 5 The presence of beacon may be accomplished in a number of ways such as by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4, 424,279 and 4,018,653. These, of course, includes both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a
10 labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid
15 substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-beacon complex, a second antibody specific to the beacon, labelled with a reporter molecule capable of producing a detectable signal, is then added and incubated, allowing time sufficient for the formation of another complex of antibody-beacon-labelled antibody. Any
20 unreacted material is washed away, and the presence of the beacon is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound
25 antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain beacon including cell extract, tissue biopsy or possibly serum, saliva, mucosal secretions, lymph, tissue fluid and respiratory fluid. The sample is, therefore, generally a biological sample comprising biological fluid but also extends to fermentation fluid and
30 supernatant fluid such as from a cell culture.

The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally
5 consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes or overnight if more convenient) and under suitable conditions (e.g. from room temperature to about 37°C) to allow binding of any subunit present in the antibody. Following
10 the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of beacon. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to beacon.

An alternative method involves immobilizing the target molecules in the biological sample and
15 then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The
20 complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most
25 commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a
30 wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-

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galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield
5 a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further
10 quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. A "reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically
15 coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic color visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the
20 unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

- 30 -

The present invention also contemplates genetic assays such as involving PCR analysis to detect *beacon* or its derivatives.

The assays of the present invention may also extend to measuring *beacon* or beacon in
5 association with *ob* or leptin.

The present invention is further described by reference to the following non-limiting Examples.

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EXAMPLE 1

Animals

A *Psammomys obesus* colony is maintained at Deakin University, with the breeding pairs fed
5 *ad libitum* a diet of lucerne and chow. Experimental animals were weaned at four weeks of age
and given a diet of standard laboratory chow from which 12% of energy was derived from fat,
63% from carbohydrate and 25% from protein (Barastoc, Pakenham, Australia). Animals were
housed individually in a temperature controlled room ($22 \pm 1^\circ\text{C}$) with a 12-12-hour light-dark
cycle. The animals used in the study were aged 16-20 weeks during the pretreatment period.
10 A total of 30 *Psammomys obesus* were investigated in this study, of which 10 were treated with
leptin as described below and 20 were used as controls (treated with saline only).

EXAMPLE 2

Leptin Treatment

15

A group of lean and obese *Psammomys obesus* were followed for a 7-day period with free
access to food and water to establish baseline data for food intake (measured by the rate of
disappearance), body weight, blood glucose and plasma insulin concentrations. After the
baseline period, the animals were given intraperitoneal injections three times per day (at 0800,
20 1600 and 2400) of 15 mg leptin per kg body weight, or equivalent volume of saline for control
animals, for a total of 7 days. This dosage of leptin resulted in a total of 45 mg/kg/day. Body
weight and food intake were measured daily throughout the study. In addition, blood was
collected from the animals on days 2, 4 and 7 at midday (the midpoint between the morning and
afternoon injections) for biochemical analyses. The results clearly demonstrated that leptin was
25 effective in reducing body weight and food intake in the lean animals, however, the obese
animal remained leptin resistant and demonstrated no differences in food intake or body weight
(Walder et al 1997b).

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EXAMPLE 3**Nicotine treatment**

As described above for the leptin treatment, animals were followed for a 1 week run in period
5 before being allocated to either infusion with 12mg/day nicotine or vehicle control infusion for
7 days. All infusions were *via* mini-osmotic pumps implanted subcutaneously (Alza, California,
USA). Nicotine treatment resulted in a significant reduction in food intake and body weight,
this effect was more pronounced than the effect of leptin described above and occurred in both
lean and obese animals.

10

At the completion of either study the animals were killed by anaesthetic overdose (120 mg/kg
pentobarbitone) and selected fat depots (interscapular, perirenal, epididymal, mesenteric and
intramuscular) were removed and weighed to allow an estimate of body fat content. The
weights of the various fat depots were combined and divided by total body mass to provide this
15 estimate.

All of the experiments described above were carried out following the Australian NHMRC
principles of laboratory animal care and approved by the Deakin University Animal Ethics
Committee, Deakin University, Geelong.

20

EXAMPLE 4**Analytical methods**

Whole blood glucose was measured using an enzymatic glucose analyser (Model 27, Yellow
25 Springs Instruments, Ohio). Plasma insulin concentrations were determined using a double
antibody solid phase radioimmunoassay (Phadeseph, Kabi Pharmacia Diagnostics, Sweden).

EXAMPLE 5

Differential display polymerase chain reaction (ddPCR)

mRNA was extracted from the hypothalamus using a Dynabeads mRNA DIRECT kit (Dyna, Oslo, Norway). The mRNA was reverse transcribed to form cDNA using the oligo-dT primer attached to the beads and AMV reverse transcriptase (Promega, Madison, WI). The ddPCR procedure developed by Liang and Pardee (1992) was modified such that second strand cDNA was produced using arbitrary 13mers and then used for the PCR reaction with the same arbitrary primer and three one-base-anchored oligo-dT primers. All primers were obtained from GenHunter Corporation (Nashville, Tennessee). The sequence of the primers that gave the beacon gene PCR product were 5'-AAGCTTTTTTTTTTTTG-3' [SEQ ID NO:4] (G-anchored primer) and 5'-AAGCTTCGGGTAA-3' [SEQ ID NO:5] (arbitrary primer 11). The 20 μ l second strand cDNA synthesis reaction contained 200 nM arbitrary primer, 12.5 μ M dNTPs, 100 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin and 1 unit Taq DNA polymerase (Qiagen, Hilden, Germany). Denaturation was performed at 94°C for one minute, annealing at 40°C for 2 minutes and elongation at 72°C for 5 minutes. The reactions were then placed at 94°C for 2 minutes to separate the 2 strands of cDNA and the second strand removed after drawing the first strand attached to magnetic beads to the side of the tube with the use of a magnet. PCR was performed using 2 μ l second strand cDNA, 200 nM of each primer, 2 μ M dNTPs, 0.2 μ l α -[³³P]dATP (2,000 Ci/mmol), 100 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% w/v gelatin and 1 unit Taq DNA polymerase (Qiagen) in a 20 μ l reaction. Amplification was carried out in a Perkin Elmer 9700 DNA thermal cycler for 40 cycles of denaturation at 94°C for 30 seconds, annealing at 40°C for 2 minutes and extension at 72°C for one minute, with a final extension at 72°C for 5 minutes. The PCR products were separated on a 4.5% w/v polyacrylamide gel, and differentially expressed PCR fragments were visualized by exposing the dried gel to x-ray film.

EXAMPLE 6

Band recovery, cloning and sequencing

Candidate bands were excised from the gel and reamplified by PCR using the appropriate
5 primer combination under the PCR conditions stated above except that the dNTP condition was
20 μ M and no radioisotope was included. The putative differentially expressed cDNA
fragments were cloned using the pCR-TRAP cloning system (GenHunter Corporation).
Sequencing reactions were carried out using ABI PRISM dye terminator cycle sequencing
ready reaction kits and analysed on an ABI 373A DNA sequencer. Gene database searches
10 were performed at the National Centre for Biotechnology Information using the BLAST
network service.

EXAMPLE 7

Quantitation of gene expression

15

Animals were killed by lethal overdose of pentobarbitone (120 mg/kg) and the following tissues
were removed: liver, spleen, kidney, heart, skeletal muscle (gastrocnemius), and adipose tissue
from the suprascapular, perirenal, intramuscular and mesenteric fat depots. RNA was extracted
from tissues using RNEasy kits (Qiagen, Hilden, Germany). RNA was quantitated by
20 spectrophotometry at 260 nm, and 1 μ g of RNA was then reverse transcribed at 42°C for 1 h
with 10U of AMV Reverse Transcriptase (Boehringer Mannheim, Mannheim, Germany)
according to the manufacturer's instructions. Oligonucleotide primers for the beacon gene PCR
were chosen from the sequence previously determined. Primers were also selected for the beta-
actin gene by comparing mRNA sequences from various mammals to identify highly conserved
25 regions. The primer sequences used were:

beta-actin - forward 5'- agtccgcgttaagtgaaca -3' [SEQ ID NO:6]

reverse 5'- ctccaggttcattccatcgt -3' [SEQ ID NO:7]

beacon - forward 5'-ggctacagcttcaccaccac-3' [SEQ ID NO:8]

reverse 5'-gcttgctgatccacatctgc-3' [SEQ ID NO:9]

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PCR was performed by adding 100 ng of cDNA to a reaction mix containing 10 mM Tris-HCl (pH 8.3 at 20°C), 1.5 mM MgCl₂, 50 mM KCl, 200 µM each dNTP, 100 pmol each primer and 1.25 U Taq DNA Polymerase (Qiagen). Standard PCR consisted of 30 cycles of 94°C for 0.5 min (denaturation), 58°C (beta-actin) or 53°C (*beacon*) for 0.5 min (annealing) and 72°C for 1 min (extension), with a final extension step of 72°C for 5 min. 10 µl of each PCR product was fractionated by agarose gel electrophoresis in a 2% w/v gel containing 0.5 µg/ml ethidium bromide at 6 V/cm for 90 min and photographed under ultraviolet transillumination at 302 nm. For quantitation of beacon gene expression, the linear phases of both of the above PCR's were determined empirically as 20 cycles for beta-actin and 24 cycles for *beacon*. PCR's and electrophoresis were conducted as above (for the appropriate number of cycles) and gene expression quantitated by computerised densitometry (Eagle Eye II System, Stratagene, USA). *beacon* gene expression was determined as the ratio of densities of *beacon* to beta-actin PCR products from the same tissues.

15

EXAMPLE 8

Statistical analysis

All experimental data are expressed as means \pm s.e.m. A one-way analysis of variance in combination with a Tukey's multiple comparison test was used to compare means between and within groups, and a two-sample unpaired t-test was used where appropriate. In all instances probability values of <0.05 were considered significant.

EXAMPLE 9

Identification of a body weight-related gene by ddPCR

25

To identify novel genes that are associated with regulation of energy balance, we compared the hypothalamic mRNA profile of lean and obese *Psammomys obesus*. One cDNA fragment amplified with the G-anchored primer and arbitrary primer 11 was found to be expressed in larger amounts in the obese animals. The cDNA band of approximately 400 base pairs was excised from the gel, reamplified and cloned.

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EXAMPLE 10

Nucleotide sequence

Both strands of the differentially expressed band from the obese animals were sequenced. The sequences were identical and are shown in Figure 1 the coding sequence is SEQ ID NO:1 and the complementary sequence is SEQ ID NO:3. The corresponding amino acid sequence is SEQ ID NO:2. This sequence was compared against nucleotide sequence databases and the six-frame conceptual translation products against protein sequence databases. Strong homology was found with genes from humans, mice, *Caenorhabditis elegans*, *Fasciola hepatica*, rice and *Saccharomyces cerevisiae* and weaker homology with ubiquitin and ubiquitin-like proteins. The genes in other species were not named, we called the gene *beacon*. The translation product of the *C. elegans* gene denoted as 'weak similarity to Arabidopsis thaliana ubiquitin-like protein 8' was 81% homologous with beacon and enabled the open reading frame of beacon to be determined. Beacon was found to be 73 amino acids long, the same length as the *C.elegans* gene. Both the start and stop codons were identified within the ddPCR fragment, eliminating the need to probe a cDNA library to determine the full sequence. The full amino acid sequence of beacon is shown in Figure 1 and the amino acid alignments with gene products in humans, mice, *C. elegans*, *F. hepatica*, rice and *S. cerevisiae*, and also human ubiquitin and ubiquitin-like protein 8 from *Arabidopsis thaliana* are shown in Figures 2A and B.

20

EXAMPLE 11

Analysis of protein

Analysis of the putative protein sequence using ProtParam tool indicated that beacon has a molecular weight of 8503.9 and is a stable protein with an estimated half-life of 30 hours. The protein does not have an aminoterminal signal sequence often found in proteins destined for export from the cell or for a membrane location. No nuclear targeting signal was found suggesting that beacon is not found in the nucleus. Transmembrane segments were also not found, but beacon may be a peripheral membrane protein, binding to the surface of integral membrane proteins. Beacon appears to have an intramitochondrial signal and may be located within the mitochondrial intermembrane space or the mitochondrial matrix space. Many

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proteins localized at the mitochondrial inner membrane are likely to be peripheral membrane proteins which exist as members of large membrane complexes.

EXAMPLE 12

5

PCR of the *beacon* gene

PCR primers were designed from the nucleotide sequence of *beacon* to yield a PCR product of 169 bp. These primers successfully yielded the correct size PCR product with *Psammomys obesus* hypothalamic cDNA. PCR of *Psammomys obesus* genomic DNA yielded the same size
10 product also, indicating that there are no introns within the gene. PCR was also performed on human genomic DNA and the same size product was detected, confirming that the *beacon* gene is also found in humans.

EXAMPLE 13

15

Tissue distribution of *beacon* gene expression in *Psammomys obesus*

The *beacon* gene was expressed at significant levels in all tissues tested in *Psammomys obesus* (hypothalamus, liver, adipose tissue, skeletal muscle (gastrocnemius), heart, kidney and spleen).

20

EXAMPLE 14

Hypothalamic *beacon* gene expression

Hypothalamic expression of the *beacon* gene was significantly correlated with body weight and
25 plasma insulin concentrations in *Psammomys obesus* (Figure 3). Correlation coefficients of *beacon* gene expression with body weight and plasma insulin were all markedly improved after six days of leptin administration (Figure 4). Neither Adipose tissue *beacon* gene expression or liver *beacon* gene expression were significantly correlated with body weight or circulating insulin levels (figure 5).

30

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EXAMPLE 15**Leptin treatment**

Leptin treatment resulted in an increase in beacon gene expression in the hypothalamus of the
5 group A, lean animals but no difference in the obese group B animals (figure 6). In contrast
beacon gene expression in adipose tissue was significantly reduced following leptin treatment
in lean animals and similarly unchanged in leptin resistant obese group B animals (figure 7).

EXAMPLE 16**Nicotine treatment**

10

Nicotine treatment failed to have any effects on beacon gene expression in either hypothalamus
or adipose tissue despite significant effects of nicotine treatment on body weight and food
intake (figure 8). These results suggest the effects of leptin treatment on beacon gene
15 expression are in fact, independent of body weight and leptin specific.

Those skilled in the art will appreciate that the invention described herein is susceptible to
variations and modifications other than those specifically described. It is to be understood that
the invention includes all such variations and modifications. The invention also includes all of
20 the steps, features, compositions and compounds referred to or indicated in this specification,
individually or collectively, and any and all combinations of any two or more of said steps or
features.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: INTERNATIONAL DIABETES INSTITUTE and DEAKIN UNIVERSITY

(ii) TITLE OF INVENTION: A NOVEL GENE AND USES THEREFOR-II

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: DAVIES COLLISON CAVE

(B) STREET: 1 LITTLE COLLINS STREET

(C) CITY: MELBOURNE

(D) STATE: VICTORIA

(E) COUNTRY: AUSTRALIA

(F) ZIP: 3000

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: AUSTRALIAN PROVISIONAL

(B) FILING DATE: 11-NOV-1997

(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: HUGHES, DR E JOHN L

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(C) TELEX: AA 31787

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 342 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 29..247

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTTCCAGGAG ATTACAGCTC CAGCCACA ATG ATT GAG GTG GTT TGC AAC GAC	52
Met Ile Glu Val Val Cys Asn Asp	
1 5	
CGT CTA GGA AAG AAA GTC CGC GTT AAG TGC AAC ACC GAT GAC ACC ATC	100
Arg Leu Gly Lys Lys Val Arg Val Lys Cys Asn Thr Asp Asp Thr Ile	
10 15 20	
GGG GAC TTG AAG AAA CTG ATA GCG GCC CAA ACT GGC ACT CGT TGG AAT	148
Gly Asp Leu Lys Lys Leu Ile Ala Ala Gln Thr Gly Thr Arg Trp Asn	
25 30 35 40	
AAG ATC GTT CTT AAA AAG TGG TAC ACG ATT TTT AAG GAC CAT GTA TCT	196
Lys Ile Val Leu Lys Lys Trp Tyr Thr Ile Phe Lys Asp His Val Ser	
45 50 55	
CTG GGA GAT TAT GAA ATC CAC GAT GGG ATG AAC CTG GAG CTT TAT TAC	244
Leu Gly Asp Tyr Glu Ile His Asp Gly Met Asn Leu Glu Leu Tyr Tyr	
60 65 70	
CAG TAGAGGGGAA TTCCTCCACC TTGCCCAACC TTGCTTTCCT CTCCCATGGC	297
Gln	
TCATTTAACA CTGTTGTAGA TGCTCATTTT TTTGTTAAGT GTACT	342

- 44 -

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 73 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Ile Glu Val Val Cys Asn Asp Arg Leu Gly Lys Lys Val Arg Val
 1             5             10             15
Lys Cys Asn Thr Asp Asp Thr Ile Gly Asp Leu Lys Lys Leu Ile Ala
                20             25             30
Ala Gln Thr Gly Thr Arg Trp Asn Lys Ile Val Leu Lys Lys Trp Tyr
                35             40             45
Thr Ile Phe Lys Asp His Val Ser Leu Gly Asp Tyr Glu Ile His Asp
 50             55             60
Gly Met Asn Leu Glu Leu Tyr Tyr Gln
65             70

```

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 391 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

ATGTTCAACA CAGCAGCCAT CCAAGGTCCT CTAATGTCGA GGTCTGGTGTT ACTAACTCCA      60
CCAAACGTTG CTGGCAGATC CTTTCTTTCA GGCGCAATTC ACGTTGTGGC TACTGTGGTA      120
GCCCCTGAAC TTCTTTGACT ATCGCCGGGT TTGACCGTGA GCAACCTTAT TCTAGCAAGA      180
ATTTTTTCACC ATGTGCTAAA AATTCCTGGT ACATAGAGAC CCTCTAATAC TTTAGGTGCT      240
ACCCTACTTG GACCTCGAAA TAATGGTCAT CTCCCCTTAA GGAGGTGGAA CGGTTGGAA      300
CGAAAGGAGA GGGTACCGAG TAAATTGTGA CAACATCTAC GAGTAAAAAA ACAATTCACA      360
TGAATAAAAA CTTTGATGCT GCAAAAAAAA A                                391

```

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 16 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- 45 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AAGCTTTTTT TTTTG

16

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AAGCTTCGGG TAA

13

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGTCCGCGTT AAGTGCAACA

20

- 46 -

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTCCAGGTTC ATCCCATCGT

20

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGCTACAGCT TCACCACCAC

20

- 47 -

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCTTGCTGAT CCACATCTGC

20

DATED this 11th day of November, 1997

International Diabetes Institute

AND Deakin University

by their Patent Attorneys

DAVIES COLLISON CAVE

5' GTTCCAGGAGATTACAGCTCCAGCCACA
 3' ATGTTCAACACAGCAGCCATCCAAGGTCTCTAATGTCGAGGTCGGTGT

10 20
 M I E V V C N D R L G K K V R V K C N T
 ATGATTGAGGTGGTTTGCAACGACCGTCTAGGAAAGAAAGTCCGCGTTAAGTGCAACACC
 TACTAACTCCACCAAACGTTGCTGGCAGATCCTTTCTTTTCAGGCGCAATTCACGTTGTGG

30 40
 D D T I G D L K K L I A A Q T G T R W N
 GATGACACCATCGGGGACTTGAAGAACTGATAGCGGCCCAAACCTGGCACTCGTTGGAAT
 CTACTGTGGTAGCCCCCTGAACCTCTTTGACTATCGCCGGGTTTGACCGTGAGCAACCTTA

50 60
 K I V L K K W Y T I F K D H V S L G D Y
 AAGATCGTTCTTAAAAAGTGGTACACGATTTTTTAAGGACCATGTATCTCTGGGAGATTAT
 TTCTAGCAAGAATTTTTCACCATGTGCTAAAAATTCCTGGTACATAGAGACCCTCTAATA

70
 E I H D G M N L E L Y Y Q STOP
 GAAATCCACGATGGGATGAACCTGGAGCTTTATTACCAGTAGAGGGGAATTCCTCCACC
 CTTTAGGTGCTACCCTACTTGGACCTCGAAATAATGGTCATCTCCCCTTAAGGAGGTGG

TTGCCCCAACCTTGCTTTCCTCTCCCATGGCTCATTTAACACTGTTGTAGATGCTCATTTTT
 AACGGGTTGGAACGAAAGGAGAGGGTACCGAGTAAATTGTGACAACATCTACGAGTAAAAA

AACCAATTCACATGAATAAAAACTTTGATGCTGCAAAAAAAA 3'
 TTGTTAAGTGTACT 5'

FIGURE 1

AMINO ACID ALIGNMENTS

A.

	10	20	30	40	50
	*	*	*	*	*
Beacon	MIEVVCNDRLGKKVRVKCNTDDTIGDLKKLIAAQTGTRWNKIVLKKWYTI				
Human	MIEVVCNDRLGKKVRVKCNTDDTIGDLKKLIAAQTGTRWNKIVLKKWYTI				
Mouse	MIEVVCNDRLGKKVRVKCNTDDTIGDLKKLIAAQTGTRWNKIVLKKWYTI				
C.elegans	MIEITVNDRLGKKVRIKCNPSDTIGDLKKLIAAQTGTRWEKIVLKKWYTI				
F.hepatica	DRLGKKVRVKCNPTDKVGDLKKLIAAQTGTAPERIVLKKWYTI				
Rice	MIEVVLNDRLGKKVRVKCNKDDTIGDLKKLVAAQTGTRPEKIRIQKWYNI				
S.cerev	MIEVVVNDRLGKKVRVKCLGEDSVGDFKKVLSLQIGTQPNKIVLQKGSV				

	60	70
	*	*
Beacon	FKDHVSLGDYEIHDGMNLELYYQ	
Human	FKDHVSLGDYEIHDGMNLELYYQ	
Mouse	FKDHVSLGDYEIHDGMNLELYYQ	
C.elegans	YKD HITLMDYEIHEGFNFELY YQ	
F.hepatica	YKDHVTLRDYEINDGMNLELYYQ	
Rice	YKD HITLADYEIHDGMGLELYYN	
S.cerev	LKD HICLEDYE VHDQTNLELYYL	

Percentage homologies

Human	73/73 = 100%
Mouse	73/73 = 100%
C.elegans	59/73 = 81%
F.hepatica	54/66 = 82%
Rice	58/73 = 79%
S.cerevisiae	46/73 = 63%

FIGURE 2A

B.

Human ubiquitin

	10	20	30	40	50
	*	*	*	*	*
Beacon	MIEVVCNDRLGKKVRVKCNTDDTIGDLKKLIAAQTGTRWNKIVLKKWYTI				
		+ ++	++	+	++
Ubiquitin	MQIFVKT LTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQ				
	60	70			
	*	*			
Beacon	FKDHVSLGDYEIHDGMNLELYYQ				
	+	+ +			
Ubiquitin	LEDGRTLSDYNIQKESTLHLVLRRLRGG				

Amino acid homology 18/73 = 25%

Positives (similar amino acids) 29/73 = 40%

Ubiquitin-like protein 8 (*A. thaliana*)

	10	20	30	40	50
	*	*	*	*	*
Beacon	MIEVVCNDRLGKKVRVKCNTDDTIGDLKKLIAAQTGTRWNKIVLKKWYTI				
	+ ++	+ ++ +	+	++ +	+
<i>A. thaliana</i>	GKTIILEVESSDTIANVKEKIQVKEGIKPDQQMLIFFGQQ				
	60	70			
	*	*			
Beacon	FKDHVSLGDYEIHDGMNLELYYQ				
	+	+ +			
<i>A. thaliana</i>	LEDGVTLGDYDIHKKSTLYL				

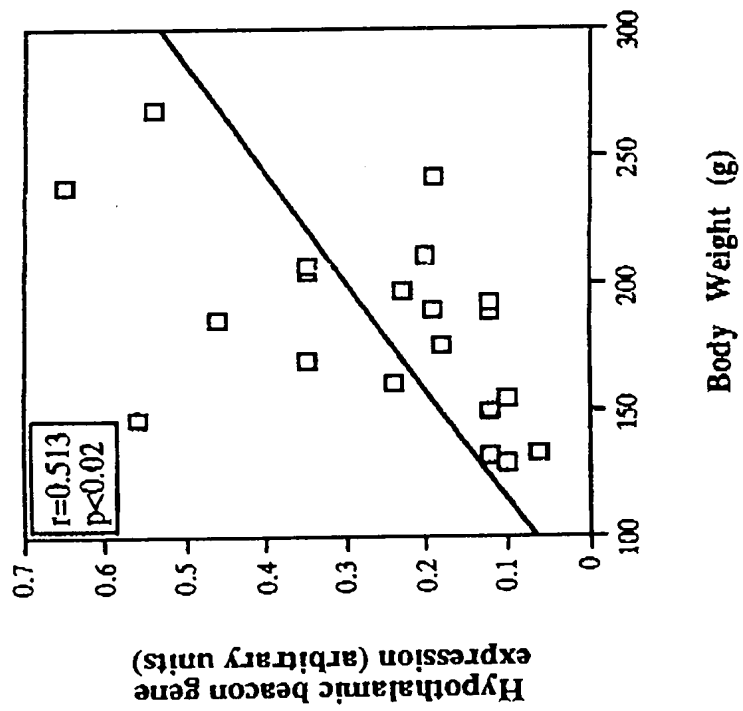
Amino acid homology 19/60 = 32%

Positives (similar amino acids) 34/60 = 57%

FIGURE 2B

FIGURE 3

A



B

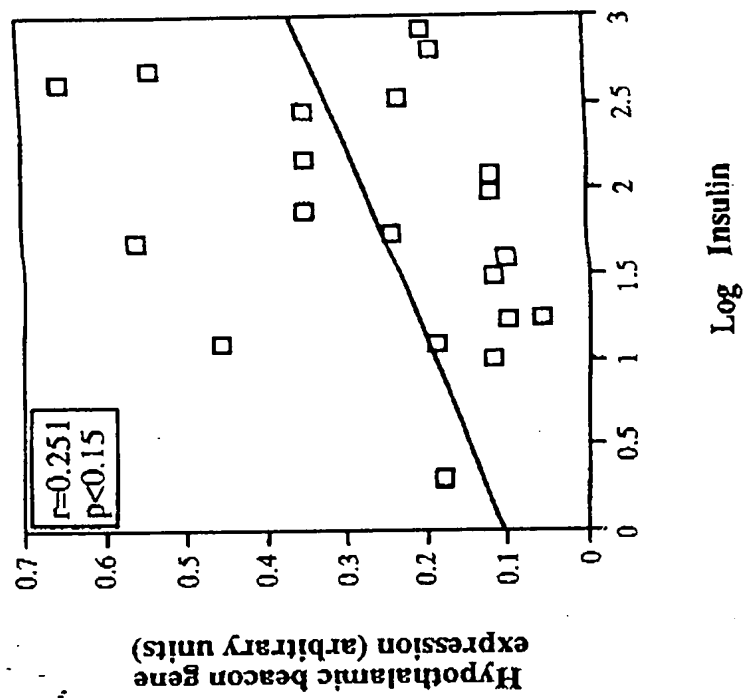
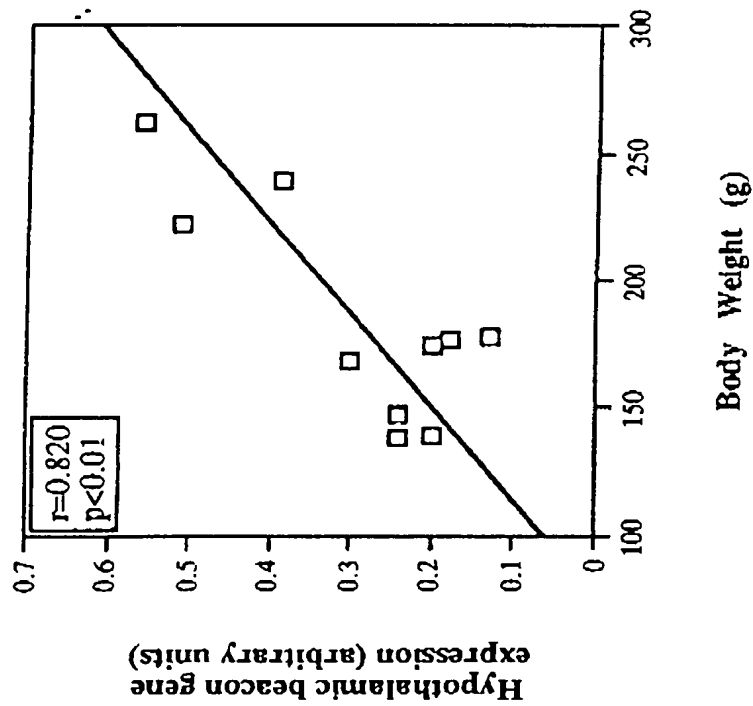


FIGURE 4

A



B

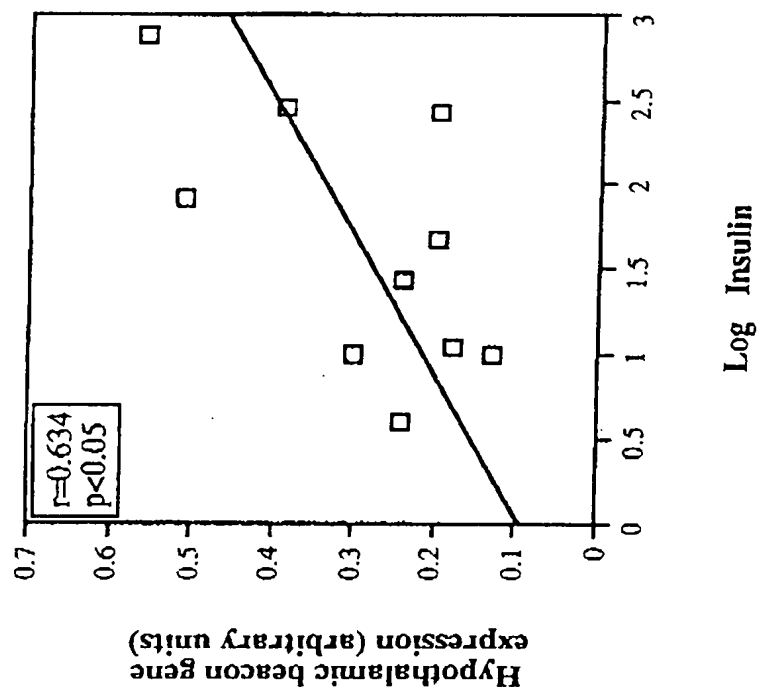
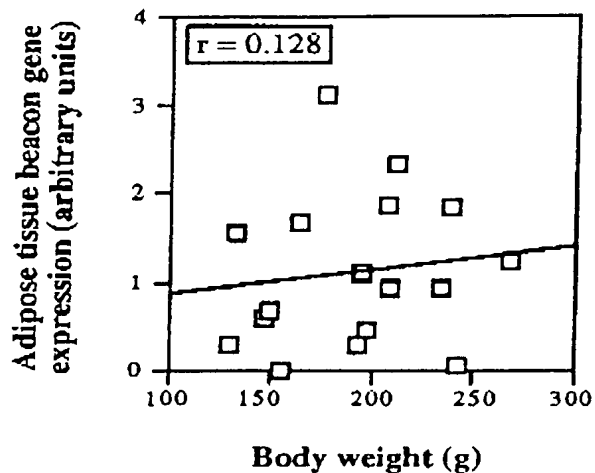
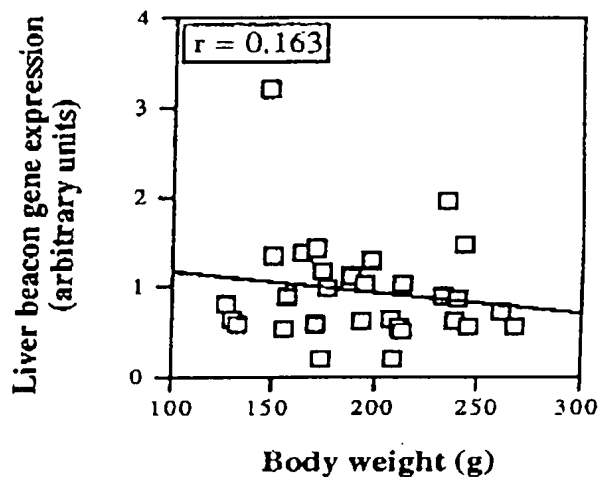


FIGURE 5

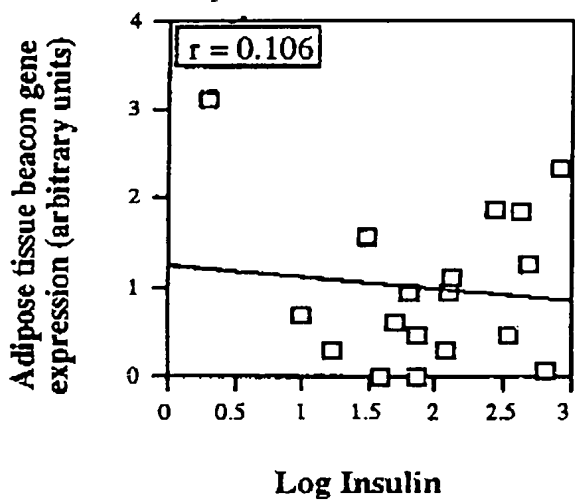
A



C



B



D

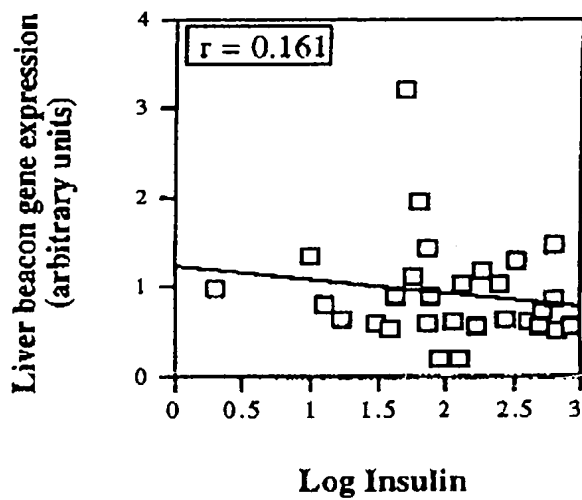


FIGURE 6

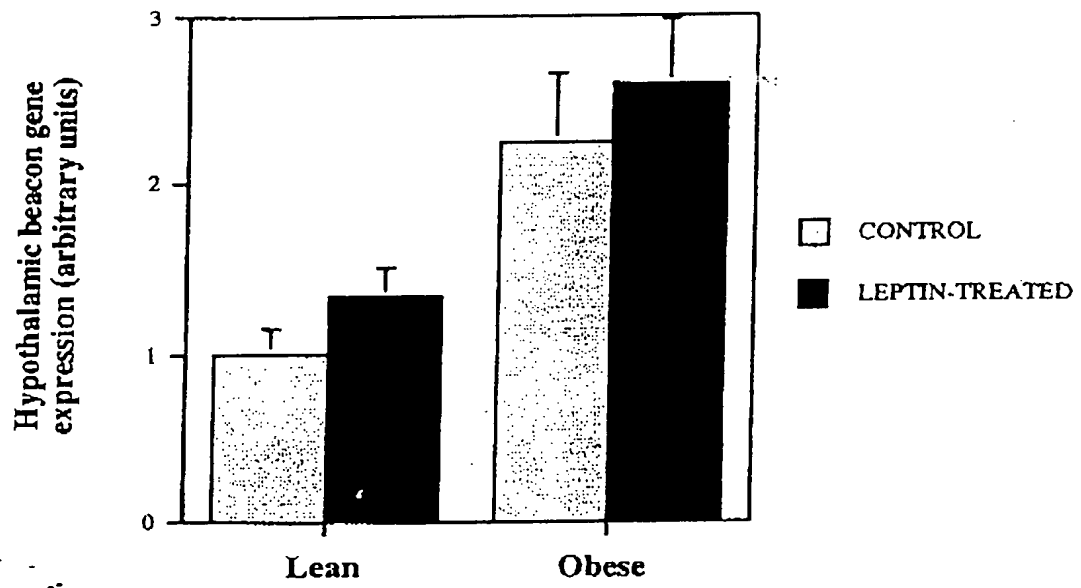


FIGURE 7

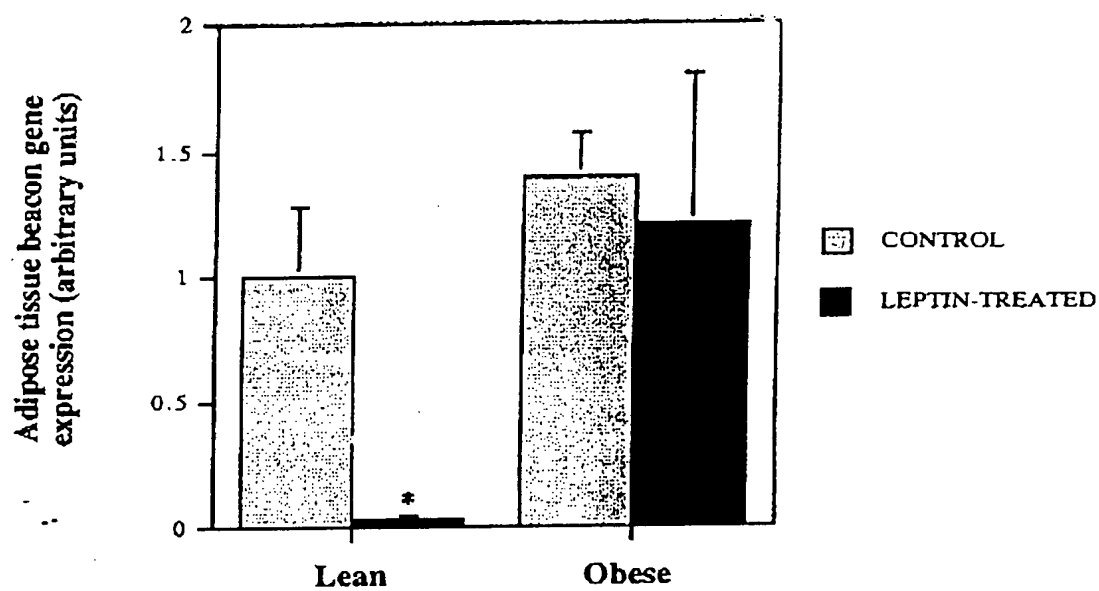
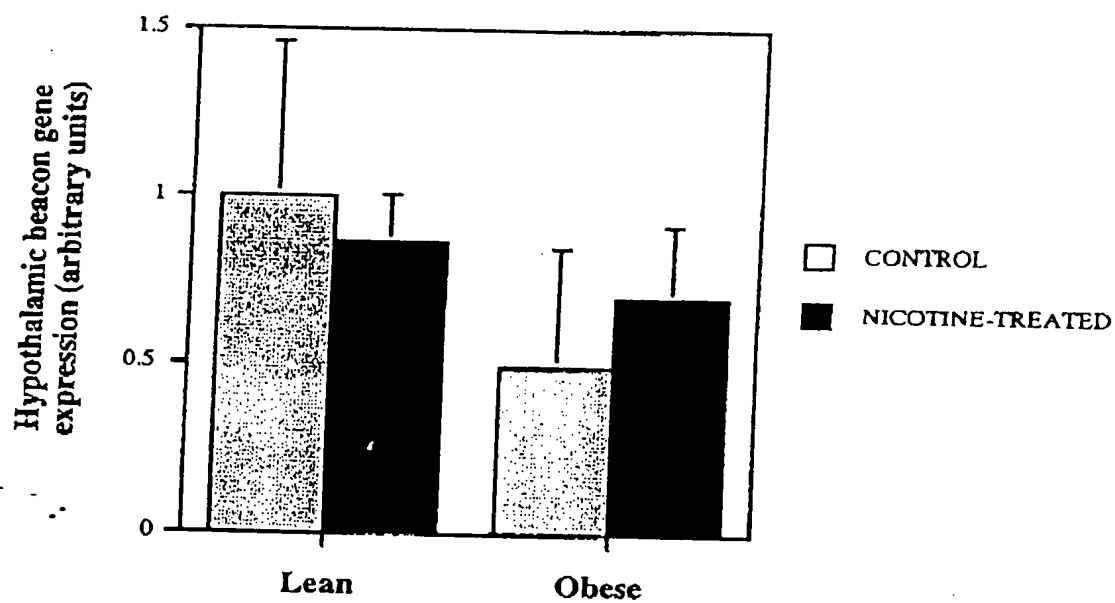
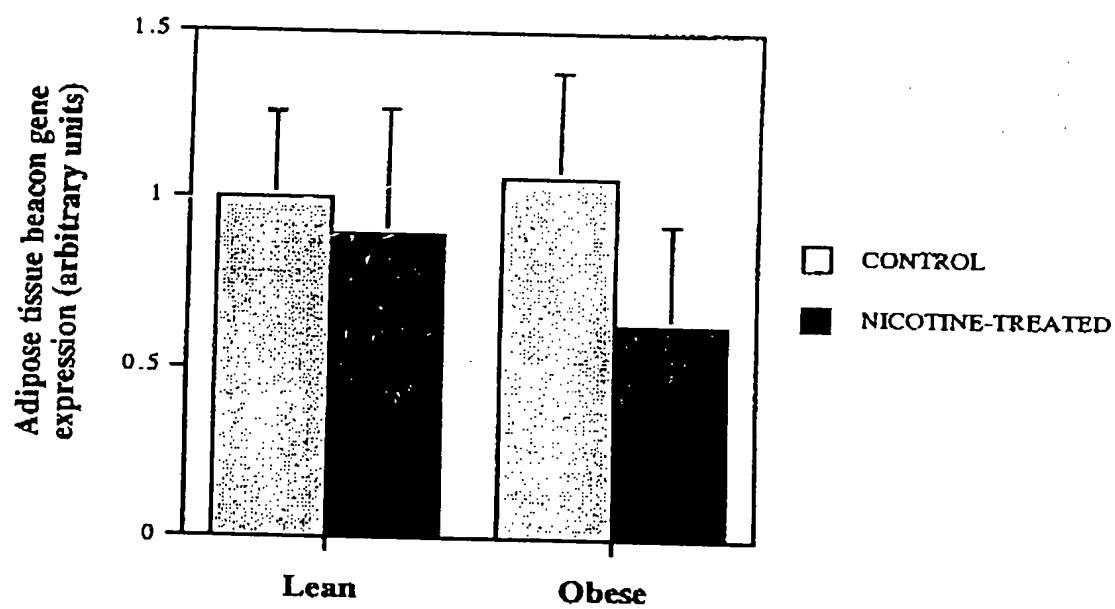


FIGURE 8

A



B



PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

HUGHES, E., John, L.
Davies Collison Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

TUESDAY, 25 MAY 1999

Date of mailing (day/month/year) 14 May 1999 (14.05.99)		
Applicant's or agent's file reference 21118060/EJH		IMPORTANT NOTICE
International application No. PCT/AU98/00902	International filing date (day/month/year) 30 October 1998 (30.10.98)	Priority date (day/month/year) 31 October 1997 (31.10.97)
Applicant INTERNATIONAL DIABETES INSTITUTE et al		

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
AU,CN,EP,IL,JP,KP,KR,US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CU,CZ,DE,DK,EA,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,
ID,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,OA,PL,PT,RO,RU,SD,SE,
SG,SI,SK,SL,TJ,TM,TR,TT,UA,UG,UZ,VN,YU,ZW

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
14 May 1999 (14.05.99) under No. WO 99/23217

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a **demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

<p>The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland</p> <p>Facsimile No. (41-22) 740.14.35</p>	<p>Authorized officer J. Zahra</p> <p>Telephone No. (41-22) 338.83.38</p>
---	---